Electronic Supplementary Information (ESI)

A carbon nanotubes/quantum dots based photoelectrochemical biosensing platform for the direct detection of microRNAs

Huijuan Cao, Shanshan Liu, Wenwen Tu,* Jianchun Bao and Zhihui Dai*

Jiangsu Collaborative Innovation Center of Biomedical Functional Materials and Jiangsu Key Laboratory of Biofunctional Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, P. R. China

*Corresponding author. Fax: +86-25-85891051; Tel: +86-25-85891051; E-mail: daizhihuii@njnu.edu.cn; wwt@njnu.edu.cn

1. Experimental Section

1.1 Reagents and materials. Single-walled carbon nanotubes (SWCNTs, φ= 10−20 nm) were purchased from Nanotech Port Co., Ltd. (Shenzhen, China). Cadmium chloride ($CdCl_2·2.5H_2O$) was purchased from Shanghai Reagent Co., Ltd. (Shanghai, China). $Na_2S·9H_2O$ was obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Thioglycolic acid (TGA) (97%) and (3-Aminopropyl) triethoxysilane (APTES) were purchased from Alfa Aesar. 1-Ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), ascorbic acid (AA) were obtained from Sigma-Aldrich. Other chemicals were of analytical reagent grade. The pH value of the phosphate buffered saline (PBS) was 7.4, except where otherwise indicated. PBS, 0.1 M, was employed as the supporting electrolyte. The washing solution was 0.01 M PBS. The blocking solution was 0.01 M PBS containing 0.1% Tween 20 (BIO-RAD). Double-

distilled water (DDW) was used in all assays. Diethylpyrocarbonate (DEPC)-treated deionized water were obtained from TaKaRa Biotechnology Co., Ltd. The oligonucleotide probe was purchased from Sangon Biological Engineering Technology & Co., Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. The synthetic miRNAs were purchased from GenePharma Company (Shanghai, China) and purified using high-performance liquid chromatography.

Phosphorothiolate phosphate (ps-po) DNA sequence:

5'- AAC TAT ACA ATC TAC TAC CTC AAA AAA T*C*G*G*G*C*G*T*A*C-3'

(*indicates phosphorothioate linkage)

Target sequence (miRNA-7f):

5'- UGA GGU AGU AGA UUG UAU AGUU -3'

Single-base mismatched target sequence (miRNA-7a):

5'- UGA GGU AGU AG**G** UUG UAU AGUU -3'

Three-base mismatched target sequence (miRNA-7d):

5'- **A**GA GGU AGU AG**G** UUG **C**AU AGUU -3'

1.2 Apparatus. High-resolution transmission electron microscope (HRTEM) images were taken on a JEOL-2100F apparatus at an accelerating voltage of 200 kV (JEOL, Japan). The photoelectrochemical measurements were performed with a Zahner photoelectrochemical workstation (Zahner, Germany). All experiments were carried out at room temperature using a conventional three-electrode system with a modified indium tin oxide (ITO) electrode (sheet resistance, 20-25 Ω/square) with a geometrical area of 0.25 cm^2 as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode as the reference electrode. All photoelectrochemical measurements were carried out under 405 nm irradiation at a constant potential of -0.05 V except where otherwise

indicated. PBS, 0.1 M, containing 0.1 M AA, was used as the supporting electrolyte for the photoelectrochemical measurements. The solutions were deaerated with high purity nitrogen for at least 15 min before each experiment, and a nitrogen atmosphere was maintained over the test solutions throughout the photoelectrochemical detection process. Ultraviolet-visible (UV-Vis) absorption spectra were recorded using a Cary 60 UV-Vis spectrometer (Agilent, USA). Fourier transform infrared (FTIR) spectra were acquired in the range of 4000-400 cm^{-1} using a Tensor 27 (Bruker, Germany). Electrochemical impedance spectroscopy (EIS) was carried out at an open circuit potential with an Autolab potentiostat/galvanostat PGSTAT302N (Metrohm, Netherland) controlled by Nova 1.8 software with a three-electrode system in a KCl solution (0.1 M) containing a $K_3Fe(CN)_6/K_4Fe(CN)_6$ (5.0 mM, 1:1) mixture as a redox probe from 0.1 Hz-100 kHz with a signal amplitude of 10 mV.

1.3 Synthesis of SWCNTs-COOH and DNA-CdS QDs. The suspension of SWCNTs-COOH was synthesized using a slightly modified procedure reported by Weng et. al.[S1] SWCNTs were dispersed in a concentrated HNO_3/H_2SO_4 (V/V, 1:1) mixture and then refluxed for 12 h at 55°C. The resulting suspension was centrifuged, and the sediment was washed with DDW until the pH reached 7.0, and then dried to obtain carboxylic group functionalized SWCNTs (SWCNTs-COOH). A 0.5 mg quantity of SWCNTs-COOH was added to 2 mL DDW and ultrasonicated to form a uniform and black SWCNTs-COOH suspension (0.25 $mg{\cdot}mL^{-1}$).

DNA-CdS QDs were prepared according to a previous report with some modifications.[S2-S4] Firstly, 5 mL of a $CdCl_2$ aqueous solution (1.25 mM) was mixed with TGA (3.1 μL). The pH of the solution was adjusted to 11.0 by the addition of a NaOH solution (1.0 M). Subsequently, 40 μL of a Na_2S (12.5 mM) aqueous solution was added to

the above solution (400 μL) in an Eppendorf tube (2 mL), and then a DNA solution containing nucleotides (120 nmol) was added. The reaction was conducted at 95°C for 1 h and then gradually cooled to room temperature. The cooled solution was purified by ultrafiltration using an Amicon Ultra-4 centrifugal-filter device with a MW cutoff of 10 kDa (Millipore Co.) at 4°C. Finally, the purified DNA-CdS QD solution was collected and stored at 4°C for further use.

1.4 Fabrication of the biosensor. The ITO electrode was sonicated in acetone and NaOH (1 M) in 1:1 (V/V) ethanol/water and then in water for 15 min each. Then, the ITO electrodes were silanized in an ethanol solution containing 2% APTES for 12 h at room temperature. After that, the electrode was carefully rinsed once with ethanol and twice with DDW, and then dried under an N_2 atmosphere. SWCNTs-COOH (15 μL, 0.25 mg·mL^{-1}) were placed on the pretreated ITO sheet. PBS (15 μL) containing NHS (0.005 M) and EDC (0.01 M) was placed on the above electrode for 1 h at room temperature, rinsed once with PBS and twice with DDW, and then dried under an N_2 atmosphere. The SWCNTs-COOH composite film-modified ITO electrode was prepared through amide bond formation.

Conjugation of the capture ssDNA-CdS QDs to the SWCNTs-COOH-modified electrodes was achieved via π-π stacking between ssDNA and SWCNTs-COOH. Briefly, probe ssDNA-CdS QDs in 20 mM Tris-HCl buffered saline, pH 8.0, containing 5 mM $MgCl_2$ and 50 mM NaCl, were placed on the SWCNTs-COOH modified electrodes for 2 h, then thoroughly rinsed with PBS (0.01 M) to remove any physically adsorbed ssDNA. Finally, the bare SWCNTs-COOH sites were treated with Tween 20 (0.1 wt%, 15 μL) at room temperature for 1 h to block any possible remaining active sites against nonspecific adsorption on the surface.

1.5 Hybridization and Measurement Procedures. MiRNAs hybridization was performed

by incubating a probe SWCNTs-COOH/ITO electrode with 15 μL of miRNAs hybridization solution containing various concentrations of miRNA-7f and 1 unit of DNase I for 2 h at 37°C. Then, the electrode was washed with PBS (0.01 M) to remove the un-hybridized miRNAs. The photoelectrochemical biosensor was then inserted into a solution of 0.1 M PBS containing 0.1 M AA to record the photoelectrochemical response at an applied potential of -0.05 V under 405 nm irradiation for miRNA detection.

2. Characterization

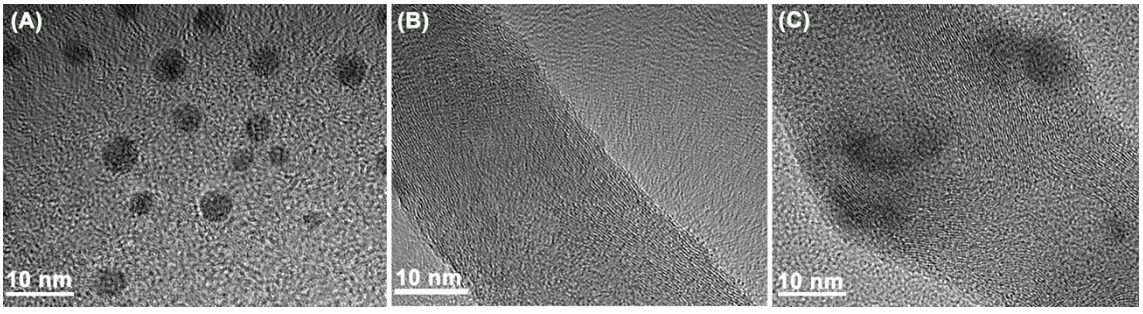

Fig. S1 HRTEM images of DNA-CdS (A), SWCNTs-COOH (B), and SWCNTs-COOH/DNA-CdS (C).

3. Photoelectrochemical response of ITO/SWCNTs-COOH/DNA-CdS/Tween 20

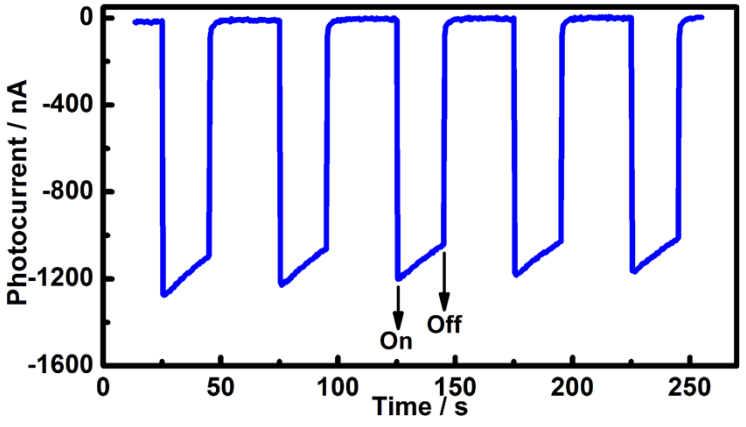

Fig. S2 Photoelectrochemical response of ITO/SWCNTs-COOH/DNA-CdS/Tween 20.

4. Optimization of the detection conditions

The excitation wavelength significantly influenced the photocurrent. At an applied potential of -0.05 V, the photocurrent decreased gradually from 365 to 405 nm, then declined sharply from 405 to 505 nm and leveled off from 505 to 530 nm (Fig. S3A). The photocurrents at 405 and 430 nm were 93.8% and 75.4% of those at 385 nm, respectively. The photocurrent at 405 nm showed enough sensitivity for photoelectrochemical detection. Moreover, a longer wavelength indicated lower energy consumption, which was favorable for monitoring biological systems. Taking these two effects into consideration, 405 nm was chosen as the excitation wavelength for use in photoelectrochemical detection.

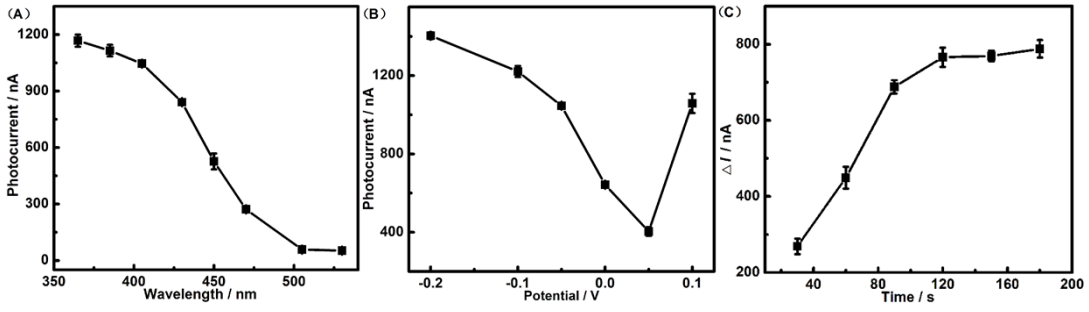

Fig. S3 Effects of (A) the excitation wavelength and (B) the applied potential on the photoelectrochemical response of the ITO/SWCNTs-COOH/DNA-CdS/Tween 20 modified ITO electrode in 0.1 M PBS containing 0.1 M AA. (C) Effect of hybridization time on the photoelectrochemical response of the biosensor toward 100 pM miRNA-7f.

The applied potential also affected the photocurrent response. At an excitation wavelength of 405 nm, as the applied potential increased from -0.2 V to 0.1 V, the cathode photocurrent decreased gradually from -0.2 to 0 V, and the anodic photocurrent increased gradually from 0.05 to 0.1 V (Fig. S3B). The cathode photocurrents at -0.05 and 0 V were 85.7% and 52.7% of those at -0.1 V, respectively. Because the photocurrent at -0.05 V was

adequately sensitive for photoelectrochemical detection, the low applied potential was beneficial for the elimination of interference from other species coexisting in the samples. Therefore, -0.05 V was selected for use in the photoelectrochemical measurements.

The hybridization time was a key factor in photoelectrochemical detection, as shown in Fig. S3C. The photocurrent responses increased rapidly with increasing incubation time from 30 to 120 min and subsequently reached a plateau with extended hybridization times. Consequently, 120 min was chosen as the hybridization time for photoelectrochemical biosensing.

Table S1. Comparison of the performance of the proposed strategy with other methods.

Method	Detection limit (M)	Linear range (M)	Reference
Electrochemistry	6×10^{-14}	1×10^{-13} - 7×10^{-11}	S5
Electrochemistry	2×10^{-13}	5×10^{-13} - 4×10^{-10}	S6
Colorimetric	1×10^{-8}	5×10^{-8} - 1×10^{-6}	S7
Fluorescence	9×10^{-12}	2×10^{-11} - 1×10^{-9}	S8
Fluorescence	3×10^{-13}	1×10^{-12} - 1×10^{-8}	S9
Photoelectrochemistry	3.4×10^{-14}	5×10^{-14} - 1×10^{-10}	this work

References

S1 Q. X. Wang, B. Zhang, X. Q. Lin and W. Weng, *Sens. Actuator B-Chem*., 2011, **156**, 599.

S2 C. L. Zhang, J. Xu, S. M. Zhang, X. H. Ji and Z. K. He, *Chem. Eur. J*., 2012, **18**,

8296.

S3 G. Tikhomirov, S. Hoogland, P. E. Lee, A. Fischer, E. H. Sargent and S. O. Kelley, *Nat. Nanotechnol*., 2011, **6**, 485.

S4 N. Ma, E. H. Sargent and S. O. Kelley, *Nat. Nanotechnol*., 2009, **4**, 121.

S5 H. S. Yin, Y. L. Zhou, H. X. Zhang, X. M. Meng and S. Y. Ai, *Biosens. Bioelectron*., 2012, **33**, 247.

S6 Z. Gao and Y. H. Yu, *Biosens. Bioelectron*., 2007, **22**, 933.

S7 Y. Zhang, Z. Li, Y. Cheng and X. Lv, *Chem. Commun*., 2009, 3172.

S8 L. Cui, X. Y. Lin, N. H. Lin, Y. L. Song, Z. Zhu, X. Chen and C. J. Yang, *Chem. Commun*., 2012, **48**, 194.

S9 Q. Xi, D. M. Zhou, Y. Y. Kan, J. Ge, Z. K. Wu, R. Q. Yu and J. H. Jiang, *Anal. Chem*., 2014, **86**, 1361.